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(54) Title: NOVEL CANNABIMIMETIC LIGANDS

(57) Abstract: Disclosed are heterocyclic compounds and methods for their manufacture. The disclosed compounds are surprisingly potent and selective cannabinoids. Also disclosed are methods of using the disclosed compounds, including use of the disclosed compounds to stimulate a cannabinoid receptor, to provide a physiological effect in an animal or individual and to treat a condition in an animal or individual.

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NOVEL CANNABIMIMETIC LIGANDS

Field of the Invention

The present invention relates generally to compounds exhibiting
5 cannabimimetic activity and is more particularly concerned with new and improved
cannabimimetic compounds exhibiting preferentially high binding affinities for the
CB2 cannabinoid receptor, methods of preparation of such compounds,
pharmaceutical preparations employing these compounds and methods of
administering therapeutically effective amounts of these compounds to provide a
10 physiological effect.

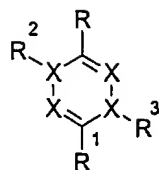
Background of the Invention

Classical cannabinoids such as the marijuana derived cannabinoid
 Δ^9 -tetrahydrocannabinol, (Δ^9 -THC) produce their pharmacological effects through
15 interaction with specific cannabinoid receptors in the body. So far, two
cannabinoid receptors have been characterized: CB1, a central receptor found in the
mammalian brain and peripheral tissues and CB2, a peripheral receptor found only
in the peripheral tissues. Compounds that are agonists or antagonists for one or
both of these receptors have been shown to provide a variety of pharmacological
20 effects.

There is considerable interest in developing new cannabimimetic compounds
possessing preferentially high affinity for the CB2 receptor. Such compounds that
preferentially stimulate the CB2 receptor, directly or indirectly, can provide clinically
useful effects without major effects on the subject's central nervous system and
25 can offer a rational therapeutic approach to a variety of disease states.

Summary of the Invention

It has now been found that certain chemical compounds surprisingly possess
cannabimimetic properties. Broadly, in one aspect of the invention the novel
30 cannabimimetic compounds can be represented by the structural Formula I,
physiologically acceptable salts, diastereomers, enantiomers, double bond isomers
or mixtures thereof.



Formula I

wherein:

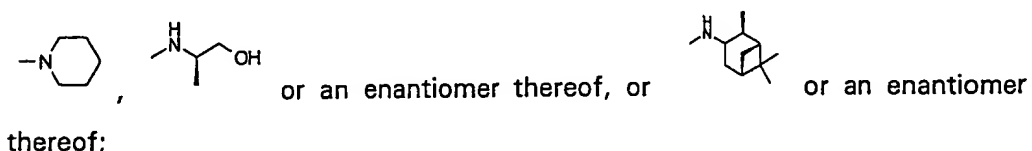
R comprises C₁₋₆alkoxy; N-alkyl; S-alkyl; C₁₋₃haloalkoxy; C₁₋₆alkylketo; C₁₋₆alkylthioketo; CO₂H; CONR⁶R⁷ where R⁶ and R⁷ each independently comprise H,
 5 lower alkyl and carbalkoxyloweralkyl; ester; thioester; reversed ester; reversed thioester; reversed amide or $\text{R}^4\text{C}(=\text{O})$ wherein R⁴ comprises

methoxy, ethoxy, propoxy, methyl, amino, methylamino, ethylamino, butylamino,



R¹ comprises C₁₋₆alkoxy; N-alkyl; S-alkyl; C₁₋₃haloalkoxy; C₁₋₆alkylketo; C₁₋₆alkylthioketo; CO₂H; CONR⁶R⁷ where R⁶ and R⁷ each independently comprise H,
 15 lower alkyl and carbalkoxyloweralkyl; ester; thioester; reversed ester; reversed thioester; reversed amide; or $\text{R}^5\text{C}(=\text{O})$ wherein R⁵ comprises

methoxy, ethoxy, propoxy, methyl, amino, methylamino, ethylamino, butylamino,



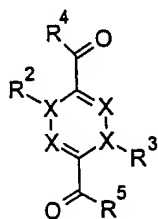
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R² and R³ each independently comprise phenyl; benzyl; α-naphthyl; methylene-α-naphthyl; β-naphthyl; methylene-β-naphthyl; 5 or 6 membered heteroaromatic rings comprising 1 to 3 heteroatoms each independently selected from N, O, and S, provided that no more than 1 heteroatom is O or S; methylene-
 30 5 or 6 membered heteroaromatic rings comprising 1 to 3 heteroatoms each independently selected from N, O, and S, provided that no more than 1 heteroatom

is O or S; any of the above comprising up to 3 substituents independently selected from halo, hydroxyl, amino, lower alkyl amino, C₁₋₆alkyl, C₁₋₆alkoxy, C₁₋₆alkylthio, CN, CF₃, CO₂H, CONR⁶R⁷ where R⁶ and R⁷ each independently comprise H, lower alkyl or carbalkoxyloweralkyl, SO₃H, and SO₂NR⁶R⁷ where R⁶ and R⁷ each
 5 independently comprise H, lower alkyl or carbalkoxyloweralkyl; terpenes; C₁₋₁₀alkyl; 1,1-dimethyl alkyl or alkoxy; and

each X independently comprises CH or N to yield either carbocyclic rings or heterocyclic rings. It should be understood that when each X is CH, the invention
 10 in any aspect encompasses the corresponding benzene derivatives i.e. 1,4-dihydrobenzenes.

In another aspect of the invention a preferred novel cannabimimetic compound can be represented by structural Formula II,


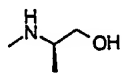


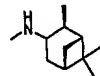
Formula II

wherein:

15 X comprises N;

R⁴ and R⁵ each independently comprise methoxy, ethoxy, propoxy, methyl,

amino, methylamino, ethylamino, butylamino, , , or an enantiomer

thereof, or  or an enantiomer thereof; and



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R² and R³ each comprise phenyl.

In another aspect of the invention a preferred novel cannabimimetic compound can be represented by structural Formula II wherein:

X comprises N;

5 R⁴ comprises ethoxy;

R⁵ comprises methyl,  or  or an enantiomer thereof; and

R² and R³ each comprise phenyl.

10 In another aspect of the invention a preferred novel cannabimimetic compound can be represented by structural Formula II wherein:

X comprises N;

R⁴ and R⁵ each comprise ethoxy; and

15

R² and R³ each independently comprise *p*-NO₂ substituted phenyl, *p*-Cl substituted phenyl, *p*-Br substituted phenyl, *p*-OMe substituted phenyl, *o*, *p*-dichloro substituted phenyl, 1-naphthyl or phenyl ketone.

20 In another aspect of the invention a preferred novel cannabimimetic compound can be represented by structural Formula II wherein:

X comprises N;

R⁴ and R⁵ each comprise ethoxy;

25

R² comprises phenyl; and

R³ comprises *p*-Cl substituted phenyl or 1-naphthyl.

In another aspect of the invention a preferred novel cannabimimetic compound can be represented by structural Formula II wherein:

X comprises N;

5 R^4 and R^5 each comprise ethoxy;

R^2 comprises *p*-Br substituted phenyl; and

R^3 comprises *p*-Cl substituted phenyl.

10

In another aspect of the invention a preferred novel cannabimimetic compound can be represented by structural Formula II wherein:

X comprises CH;

15 R^4 and R^5 each comprise ethoxy; and

R^2 and R^3 each comprise phenyl.

20 In another aspect of the invention a preferred novel cannabimimetic compound can be represented by structural Formula II wherein:

X comprises N;

R^4 and R^5 each comprise ethoxy; and

25 R^2 and R^3 each comprise naphthyl.

Naturally, the invention in any aspect also encompasses any of physiologically acceptable salts, diastereomers, enantiomers, double bond isomers and mixtures of the above inventive compounds. Further, when each X is CH, the
30 invention in any aspect encompasses the corresponding benzene derivatives i.e.

1,4-dihydrobenzenes. The compounds represented by structural Formula II are also encompassed within the broader invention represented by structural Formula I.

Unless otherwise specifically defined, "alkyl" refers to a linear, branched or cyclic alkyl group having from 1 to about 9 carbon atoms including, for example, methyl, ethyl, propyl, butyl, hexyl, octyl, isopropyl, isobutyl, tert-butyl, cyclopropyl, cyclohexyl, cyclooctyl, vinyl and allyl. The alkyl group can be saturated or unsaturated and substituted or unsubstituted. Unless otherwise specifically defined, "lower-alcohol" refers to the general formula alkyl-OH. Unless otherwise specifically defined, "alkoxy" refers to the general formula -O-alkyl. Unless otherwise specifically defined, "alkylmercapto" refers to the general formula -S-alkyl. Unless otherwise specifically defined, "alkylamino" refers to the general formula -(NH)-alkyl. Unless otherwise specifically defined, "di-alkylamino" refers to the general formula -N-(alkyl)₂. Unless otherwise specifically defined, an aromatic ring is an unsaturated ring structure, substituted or unsubstituted, that includes only carbon as ring atoms. Unless otherwise specifically defined, a heteroaromatic ring is an unsaturated ring structure, substituted or unsubstituted, that has carbon atoms and one or more heteroatoms, including oxygen, nitrogen and/or sulfur, as ring atoms, for example, pyridine, furan, quinoline, and their derivatives. Unless otherwise specifically defined, a carbocyclic ring is a saturated ring structure, substituted or unsubstituted, that includes only carbon as ring atoms, for example, cyclohexane. Unless otherwise specifically defined, a heterocyclic ring is a saturated ring structure, substituted or unsubstituted, that has carbon atoms and one or more heteroatoms, including oxygen, nitrogen and/or sulfur, as ring atoms, for example, piperidine, morpholine, piperazine, and their derivatives. Unless otherwise specifically defined, a terpene is an unsaturated hydrocarbon having the general formula C₁₀H₁₆ and based on the isoprene (C₅H₈) unit. As used herein a terpene may be acyclic, monocyclic or polycyclic and substituted or unsubstituted.

Substituent groups for the above moieties useful in the invention are those groups that do not significantly diminish the biological activity of the inventive

compound. Unless otherwise specifically defined, substituent groups that do not significantly diminish the biological activity of the inventive compound include, for example, -OH, -NH₂, alkoxy, halogen, -CF₃, -CN, -NCS, azido, -CONH, -NHCO, sulfonamide, lower alcohol.

5 Some of the inventive cannabinoid compounds exhibit high affinity for the CB2 cannabinoid receptor. Thus, another aspect of the invention is use of at least one of the inventive compounds to interact with the CB2 cannabinoid receptor.

Further, some of the inventive cannabinoid compounds show a surprisingly higher selectivity for the CB2 cannabinoid receptor. These inventive selective
10 compounds are able to interact with the CB2 cannabinoid receptor, without affecting the CB1 cannabinoid receptor to the same degree. Therefore, still another aspect of the invention is use of at least one of the inventive compounds to preferentially interact with the CB2 cannabinoid receptor.

Some of the inventive cannabinoid compounds can act as high affinity
15 modulators for the CB2 cannabinoid receptor. The inventive cannabinoid compounds therefore are potential therapeutic agents through the modulation of the CB2 cannabinoid receptor.

Some of the novel cannabinoid compounds described herein may be agonists for the CB2 cannabinoid receptor. The inventive cannabinoid agonists interact with
20 the CB2 cannabinoid receptor binding site to initiate a physiological or a pharmacological response characteristic of that receptor. Therefore, a further aspect of the invention is use of at least one of the inventive compounds to initiate an agonistic response from a CB2 cannabinoid receptor.

The inventive cannabinoid compounds described herein, and physiologically
25 acceptable salts thereof, have pharmacological properties when administered in therapeutically effective amounts for providing a physiological response in individuals and/or animals. Thus, another aspect of the invention is the administration of a therapeutically effective amount of at least one of the inventive cannabimimetic compounds, or a physiologically acceptable salt thereof, to an
30 individual or animal to provide a physiological response.

The inventive cannabinoid compounds have uniquely short and simple

synthesis routes. Thus another aspect of the invention are methods of preparation of the inventive cannabinoid compounds.

A better understanding of the invention will be obtained from the following detailed description of the article and the desired features, properties, characteristics, and the relation of the elements as well as the process steps, one with respect to each of the others, as set forth and exemplified in the description and illustrative embodiments.

Description of a Preferred Embodiment

As used herein a "therapeutically effective amount" of a compound, is the quantity of a compound which, when administered to an individual or animal, results in a sufficiently high level of that compound in the individual or animal to cause a discernible increase or decrease in stimulation of cannabinoid receptors. The discernible increase or decrease in stimulation of cannabinoid receptors provides a physiological response in the individual or animal. The inventive compounds described herein, and physiologically acceptable salts thereof, have pharmacological properties when administered in therapeutically effective amounts for providing a physiological response useful to: treat pain, peripheral pain, glaucoma, epilepsy and nausea such as associated with cancer chemotherapy; cancer, especially glioma and breast cancer; neurodegenerative diseases including Multiple Sclerosis, Parkinson's Disease, Huntington's Chorea and Alzheimer's Disease, reduce fertility; prevent or reduce diseases associated with motor function such as Tourette's syndrome; prevent or reduce inflammation; provide neuroprotection; to modulation of the immune system; or treat a combination of the above. Typically, a "therapeutically effective amount" of the novel cannabimimetic compounds may range from about 10 mg/day to about 1,000 mg/day.

As used herein, an "individual" refers to a human. An "animal" refers to, for example, veterinary animals, such as dogs, cats, horses and the like, and farm animals, such as cows, pigs and the like.

The compound of the present invention can be administered by a variety of known methods, including orally, rectally, or by parenteral routes (e.g.,

intramuscular, intravenous, subcutaneous, nasal or topical). The form in which the compounds are administered will be determined by the route of administration. Such forms include, but are not limited to, capsular and tablet formulations (for oral and rectal administration), liquid formulations (for oral, intravenous, intramuscular or subcutaneous administration) and slow releasing microcarriers (for rectal, intramuscular or intravenous administration). The formulations can also contain a physiologically acceptable vehicle and optional adjuvants, flavorings, colorants and preservatives. Suitable physiologically to acceptable vehicles may include, for example, saline, sterile water, Ringer's solution, and isotonic sodium chloride solutions. The specific dosage level of compound will depend upon a number of factors, including, for example, biological activity of the particular preparation, age, body weight, sex and general health of the individual being treated.

The following examples are given for purposes of illustration only in order that the present invention may be more fully understood. These examples are not intended to limit in any way the scope of the invention unless otherwise specifically indicated.

Examples:

TABLE 1 illustrates some cannabinoids of the present invention (compounds 1-25).

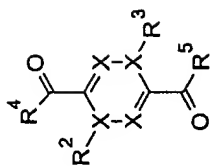

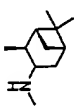
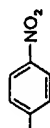
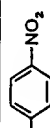
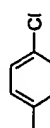
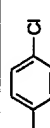
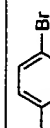
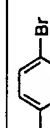
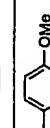
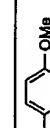
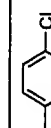
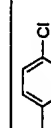
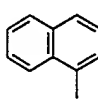
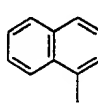
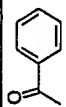
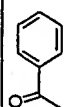
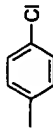

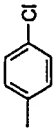
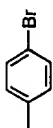






TABLE 1

compound	X	R ⁴	R ⁵	R ²	R ³	Selectivity for CB2	K _i (nM)	
							CB1	CB2
1	N	OCH ₃	OCH ₃	phenyl	phenyl	81	3.1 x 10 ⁴	381
2	N	OC ₂ H ₅	OC ₂ H ₅	phenyl	phenyl	88	1672	19
3	N	OC ₃ H ₇	OC ₃ H ₇	phenyl	phenyl	27	618	23.3
4	N	CH ₃	CH ₃	phenyl	phenyl	1	1.0 x 10 ⁴	1.0 x 10 ⁴
5	N	NH ₂	NH ₂	phenyl	phenyl	1	1.0 x 10 ⁴	1.0 x 10 ⁴
6	N	NHCH ₃	NHCH ₃	phenyl	phenyl	1	6.6 x 10 ⁴	1.0 x 10 ⁵
7	N	NHC ₂ H ₅	NHC ₂ H ₅	phenyl	phenyl	1	1.0 x 10 ⁵	1.0 x 10 ⁵
8	N	NHC ₄ H ₉	NHC ₄ H ₉	phenyl	phenyl	23	1.6 x 10 ⁴	692
9	N	N-(piperidin-2-yl)	N-(piperidin-2-yl)	phenyl	phenyl	42	9985	221
10	N	N-(1-hydroxy-2-methylpropyl)	N-(1-hydroxy-2-methylpropyl)	phenyl	phenyl	1	1.0 x 10 ⁵	1.0 x 10 ⁵
11	N	N-(1-methyl-4-phenylpiperidin-4-yl)	N-(1-methyl-4-phenylpiperidin-4-yl)	phenyl	phenyl	1	1.0 x 10 ⁵	1.0 x 10 ⁵

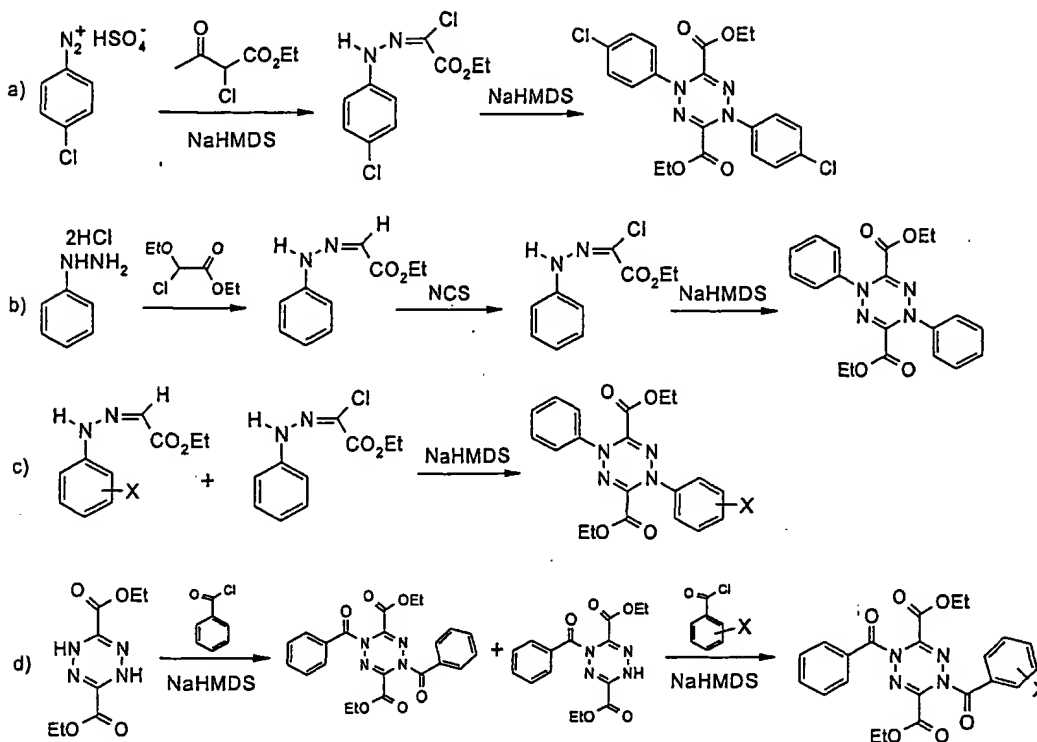
compound	X	R ⁴	R ⁵	R ²	R ³	Selectivity for CB2	K _i (nM)	
							CB1	CB2
12	N	CH ₃	OC ₂ H ₅	phenyl	phenyl	24	7704	327
13	N		OC ₂ H ₅	phenyl	phenyl	20	5044	255
14	N		OC ₂ H ₅	phenyl	phenyl	2.6	2054	805
15	N	OC ₂ H ₅	OC ₂ H ₅				1.0 x 10 ⁵	1.0 x 10 ⁵
16	N	OC ₂ H ₅	OC ₂ H ₅			4.3	208	48
17	N	OC ₂ H ₅	OC ₂ H ₅			3.5	350	200
18	N	OC ₂ H ₅	OC ₂ H ₅			64	4585	72
19	N	OC ₂ H ₅	OC ₂ H ₅			1	1.0 x 10 ⁴	1.0 x 10 ⁴
20	N	OC ₂ H ₅	OC ₂ H ₅			524	1.0 x 10 ⁸	1906
21	N	OC ₂ H ₅	OC ₂ H ₅			1	1.0 x 10 ⁵	1.0 x 10 ⁵

compound	X	R ⁴	R ⁵	R ²	R ³	Selectivity for CB2	K _i (nM)	
							CB1	CB2
22	N	OC ₂ H ₅	OC ₂ H ₅			34	1500	43
23	N	OC ₂ H ₅	OC ₂ H ₅			18	542	30
24	N	OC ₂ H ₅	OC ₂ H ₅			40	7357	182
25	C	OC ₂ H ₅	OC ₂ H ₅			1.6	1203	747

Preparation of compounds:

The materials listed in Table 1 can be prepared by following one of the methods outlined in Scheme 1.

5



Scheme 1

It has been found preferable to carry out the reactions involving sodium hexamethyldisilazane at temperatures in the range of about 25 °C to about -78 °C.

10 It should be noted that conversion of esters of the invention to their corresponding methyl ketones can be accomplished using the method of M. Girardot, R. Nomak, and J.K. Snyder; J. Org. Chem. (1998) 63(26) 10063-10068, the content of which is incorporated by reference herein. Such conversion is within the scope of the present invention.

Examples of specific analogs were prepared as follows:

Ethyl phenylhydrazinochloromethylenecarboxylate (5.6)

Method A: Concentrated sulfuric acid (50 mL, 96%) was slowly dropped into
5 a solution of aniline (30 g, 0.32 mol) in ethanol (280 mL) until the white precipitate
newly formed disappeared again at 0-5 °C. To this mixture, isoamyl nitrite (40 g,
0.34 mol) was slowly added with stirring. An equal volume of ethyl ether was
added to precipitate the product. The mixture was filtered and the residue washed
with ethanol:ether (1:1, v/v) to provide a crude greenish solid of benzenediazonium
10 sulfuric acid salt for the following reaction.

Mixed with a small amount of ice water, the above product was added to a
mixture of ethyl α -chloroacetoacetate (72.6 g, 0.44 mol), ethanol (616 mL), and
sodium acetate (54.12 g, 0.66 mol) in several portions at 0 to about 5 °C. After
stirring for 3 h, the reaction mixture was poured into a large volume of water and
15 left overnight. Collecting the precipitate by filtration provided a light brown solid
5.6 (21.72 g, overall yield 30%) with high purity. Recrystallization was performed
from ethanol to give crystal-like solid with mp 77-78 °C [lit (Bowack and Lapworth,
1905) 71-72 °C]. ¹H NMR (200MHz, CDCl₃) δ (ppm): 1.43 (t, J = 7.4 Hz, 3H),
4.39 (q, J = 7.4 Hz, 2H), 7.02-7.08 (m, 1H), 7.20-7.38 (m, 4H), 8.34 (b, 1H).
20 GC-MS (EI): 226 (M⁺), 181 (M⁺ - OCH₂CH₃), 152, 91, 65.

Method B: To a solution of ethyl phenylhydrazono acetic acid ester (5.25),
172 mg, 0.88 mmol) in ethyl acetate (5 mL) was added N-chlorosuccinimide (NCS,
129.7 mg, 1.1 mmol), and the resulting reaction mixture was heated at 60-70 °C
25 for 24 h. TLC indicated the reaction was complete. After removal of the solvent,
the crude product was subjected to silica gel column chromatography with
petroleum ether and ethyl acetate (20: 1) as eluent system to afford the title
compound in 83.3 % yield.

Ethyl naphthalenylhydrazinochloromethylenecarboxylate (5.7)

Analogously to the synthesis of 5.6, the 1-naphthalenediazonium sulfuric acid salt was first prepared and then reacted with ethyl 2-chloro acetoacetate to provide the title compound in 16.6% overall yield as red-brown solid, mp 83-84 °C (recrystallization from ethyl acetate- petroleum ether). ¹H NMR (200 MHz, CDCl₃) δ (ppm): 1.43 (t, J = 7.1 Hz, 3H), 4.43 (q, J = 7.1 Hz, 2H), 7.48-7.65 (m, 5H), 7.86-7.90 (m, 2H), 8.94 (s, 1H). ¹³C NMR δ ppm: 14.46, 63.10, 111.49, 118.16, 119.26, 122.78, 123.56, 126.41, 129.15, and 134.35, 137.10, 159.3.

10 Ethyl 4-chlorophenylhydrazinochloromethylenecarboxylate (5.12)

Using 4-chlorobenzenediazonium hexafluorophosphate as starting material and a method analogous to the synthesis of 5.6, the title compound was prepared in 61% yield as a yellow solid, mp 146-147 °C. ¹H NMR (200 MHz, CDCl₃) δ (ppm): 8.30 (s, 1H), 7.21 (d, J = 8.0 Hz, 2H), 4.40 (dd, J = 7.0 Hz, 2H), 1.39 (t, J = 7.0 Hz, 3H). GC-MS (EI), m/e: 261 (M⁺), 186, 125, 99.

Ethyl 4-bromophenylhydrazinochloromethylenecarboxylate (5.13)

Using 4-bromobenzenediazonium tetrafluoroborate as starting material, and a method analogous to the synthesis of 5.6, the title compound was prepared in 61% yield as yellow solid, mp 160-161 °C. ¹H NMR (200 MHz, CDCl₃) δ (ppm): 1.40 (t, J = 7.2 Hz, 3H), 4.39 (dd, J = 7.1 Hz, 2H), 7.11 (d, J = 8.6 Hz, 2H), 7.44 (d, J = 8.6 Hz, 2H), 8.30 (s, 1H). GC-MS (EI), no GC peak.

25 Ethyl 4-nitrophenylhydrazinochloromethylenecarboxylate (5.15)

Using 4-nitrobenzenediazonium hexafluorophosphate and ethyl 2-chloroacetoacetate as starting materials, and a method analogous to the synthesis of 5.6, the title compound was prepared in 70% yield as yellow solid, mp 193-194 °C. ¹H NMR (200 MHz, CDCl₃) δ (ppm): 1.43 (t, J = 7.2 Hz, 3H), 4.42 (q, J = 7.2 Hz, 2H), 7.32 (d, J = 7.3 Hz, 2H), 8.25 (d, J = 8.8 Hz, 2H), 8.57 (b, 1H). GC-MS (EI): no GC peak.

Acetyl phenylhydrazinochloromethylene (5.18)

Using benzenediazonium salt as starting material reaction with 3-chloro-2, 4-pentanedione (5.17) in a method analogous to the synthesis of 5-6 provided the title compound in 14% yield as brown solid, mp 129.5-130 °C. ¹H NMR (200MHz, CDCl₃) δ (ppm): 8.48 (s, 1H), 7.31 (dd, J = 8.5 Hz, 4H), 7.10 (t, J = 8.0Hz, 1H), 2.50 (s, 3H). GC-MS (EI), m/e: 196(M⁺), 118, 91, 77, 65.

Acetyl 4-chlorophenylhydrazinochloromethylene (5.19)

Using 4-chlorobenzene- diazonium hexafluorophosphate as starting material, and reacting with 3-chloro-2, 4- pentanedione (5.17) analogously to the synthesis of 5-6 provided the title compound in 92% yield as red-brown solid, mp 161-162 °C. ¹H NMR (200MHz, CDCl₃) δ (ppm): 8.45 (s, 1H), 7.31 (dd, J = 8.5 Hz, 4H), 2.57 (s, 3H). GC-MS (EI), m/e: 231(M⁺) 194, 179, 152. 125, 99.

Ethyl phenylhydrazono acetic acid ester (5.25)

Phenyl hydrazine hydrochloride (1.45 g, 10 mmol) was suspended in water (5 mL) (the resulting mixture showed pH -4). To this mixture was added a solution of 2-chloro-2-ethoxyacetate (prepared from ethyl 2, 2- diethoxyacetate and acetyl chloride without further purification) in dioxane (12.5 mL) in small portions while cooling with tap water. After: 3 h, the reaction mixture was neutralized to pH-8 with sodium hydroxide solution and evaporated under vacuum to half its volume. Water was added to the mixture and the resulting emulsion was extracted with dichloromethane. The organic layers were separated, dried over MgSO₄, filtered, and evaporated in vacuo. The crude product was subjected to recrystallization from ethyl acetate and petroleum ether to afford the title compound 5.25 as light brown solid (200 mg, 10.4%), mp 129-131 °C (lit: 130-132 °C, Jung, et al, 1982). ¹H NMR (200 MHz, CDCl₃) δ 1.36 (t, J = 7.1 Hz, 3H), 4.32 (q, J = 7.1 Hz, 2H), 5.30 (s, 1H). 6.96-7.35 (m, 5H), 8.33 (b, 1H).

1, 4-Di-(4-chlorophenyl)-1, 4-dihydro-1, 2, 4, 5-tetrazine-3, 6-dicarboxylic acid diethyl ester (5.28)

To a stirring solution of 5.12 (100 mg, 0.38 mmol) in THF was added sodiohexamethyldisilazane (0.38 mL, 1 M solution in THF) at 0 °C. The reaction mixture was warmed up to RT and stirred for another 3 h. Work-up with ammonium chloride aqueous solution and extraction with ethyl acetate, which was dried over sodium sulfate provided the crude product after removal of the solvent under vacuum. The crude product was purified by flash column chromatography to afford the title compound (31.5 mg, 37%) as an orange solid. ¹H NMR (200MHz, CDCl₃) δ 7.28 (dd, J = 8.5 Hz, 8H), 4.18 (dd, J = 7.0 Hz, 4H), 1.10 (t, J = 7.0 Hz, 6H). ¹³C NMR (200MHz, CDCl₃) δ 159.2, 141.5, 140.2, 131.0, 129.4, 120.2, 63.3, 13.8. GC-MS (EI), m/e: 449(M⁺), 376, 152, 111. Anal. (C₂₀H₁₈Cl₂N₄O₄) C, H, N.

1, 4-Di-(α-naphthalene)-1, 4-dihydro-1, 2, 4, 5-tetrazine-3, 6-dicarboxylic acid diethyl ester (5.32)

Analogously to the synthesis of compound 5.28, except that the reaction was run at -78 °C after failure at 0 °C, the title compound was prepared from 5.7 in 61% yield as a red brown solid, mp 129-130 °C recrystallization from ethyl acetate). ¹H NMR (200 MHz, CDCl₃) δ 0.66 (t, J = 7.0 Hz, 6H), 3.82 (q, J = 7.0 Hz, 4H), 7.46- 7.53 (m, 4H), 7.59 (d, J = 7.2 Hz, 2H), 7.78 (d, J = 8.0 Hz, 2H), 7.85-7.90 (m, 4H), 8.07- 8.12 (m, 2H). Anal. (C₂₈H₂₄N₄O₄) C, H, N.

1, 4-Diphenyl-1, 4-dihydro-3, 6-diacetyl-1, 2, 4, 5-tetrazine (5.33)

Analogously to the synthesis of compound 5.28, the title compound was prepared from 5.17 in 31% yield. ¹H NMR (200MHz, CDCl₃) δ 7.30 (dd, J = 8.5 Hz, 8H), 7.10 (t, J = 8.0 Hz, 2H), 2.50 (s, 6H). GC-MS (EI), m/e: 320(M⁺), 278, 207, 91, 77.

1, 4-Di-(4-chlorophenyl)-1, 4-dihydro-3, 6-diacetyl-1, 2, 4, 5-tetrazine (5.34)

Analogously to the synthesis of compound 5.28, the title compound was prepared from 5.18 in 20% yield. ¹H NMR (200MHz, CDCl₃) δ 7.28 (dd, J = 8.5 Hz, 8H), 2.49(s, 6H). GC-MS (EI), m/e: 346(M⁺ -COCH₃, 304).

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Dimethyl 1, 4-diphenyl-1, 4-dihydro-1, 2, 4, 5-tetrazine-3, 6-dicarboxylate (5.37)

Sodium cyanide (0.5 mg) was added to a solution of compound 5.1 (38 mg, 0.1 mmol) in methanol (0.5 mL) and the resulting suspension was stirred at room temperature for 12 h. After removal of solvent, the residue was dissolved in
10 dichloromethane and washed with water. The organic layer was dried over sodium sulfate and evaporated under vacuum to provide the title compound 5.37 (35 mg, 99%) as an orange solid. Recrystallization from 10% dichloromethane in ethanol afforded the title compound as an orange solid, mp 171- 172 °C. ¹H NMR (200MHz, CDCl₃) δ 7.41-7.09 (m, 10H), 3.69 (s, 6H). ¹³C NMR (200MHz, CDCl₃)
15 δ 160.0, 141.5, 129.3, 125.6, 118.7. and 53.5. Anal. (C₁₈H₁₆N₄O₄) C, H, N.

Dipropyl-1, 4-diphenyl-1, 4-dihydro-1, 2, 4, 5-tetrazine-3, 6-dicarboxylate (5.38)

The title compound was prepared in n-propanol analogously to the synthesis of 5.37 in 98% yield as an orange solid, mp 82.5-83 °C. ¹H NMR (200MHz, CDCl₃)
20 δ 7.30 (m, 10H), 4.02 (q, J = 6.0 Hz, 4H), 1.44 (t, J = 7.2 Hz, 4H), 0.75(t, J=7.2 Hz, 6H). ¹³C NMR (200MHz, CDCl₃) δ 159.8, 141.9, 141.7, 129.3, 125.5, 118.6, 68.6, 21.7, and 10.3. GC-MS (EI). m/e: 408 (M⁺), 322, 118, and 77. Anal. (C₂₂H₂₄N₄O₄) C, H, N.

25 1, 4-Diphenyl-1, 4-dihydro-1, 2, 4, 5-tetrazine-3, 6-dicarboxylic amide (5.39)

Liquid ammonia was slowly added to a suspension of 5.1 (76 mg, 0.2 mmol) and sodium cyanide (1 mg, 0.02 mmol) in methanol (2 mL) in a sealing tube at -60 to about -80 °C for 2 min. After slow warming up to -20 °C, the reaction mixture was sealed, and warmed up to room temperature. After stirring at RT for two
30 days, the reaction mixture was cooled down to -20 °C again for opening the seal, and then warmed up to RT for workup. The solvent was removed by rotary

evaporation, and the residue was dissolved in ethanol for filtration. After concentration, the filtrate gave the crude product, which was recrystallized from ethanol to provide the title compound 5.39 as a red-brown solid (18 mg, 28%). The mother liquor afforded another portion of the title compound (40 mg, 62%) after concentration and flash column purification. The overall yield is 90%, mp 241-242 °C. ¹H NMR (200MHz, CDCl₃) δ 8.43(s, 2H), 7.95(s, 2H), 7.28 (m, 8H), 7.08(t, J = 5.6Hz, 2H). ¹³C NMR (200MHz, CDCl₃) δ 160.3, 144.4, 141.3, 128.6, 124.1, and 117.8. Anal. (C₁₆H₁₄N₆O₂) C, H, N.

10 **N, N-Dimethyl-1, 4-diphenyl-1, 4-dihydro-1, 2, 4, 5-tetrazine-3, 6-dicarboxylic di-amide (5.40)**

Using methyl amine (2 M solution of methyl amine in methanol) as reactant, the title compound was prepared analogously to the synthesis of compound 5.37 in 99% yield (the reaction was finished in 4 hours) as an orange solid, mp 243-243.5 °C. ¹H NMR (200MHz, CDCl₃) δ 7.30 (m, 10H), 6.60(m, 2H), 2.83 (s, 3H), 2.81(s, 3H), ¹³C NMR (200MHz, CDCl₃) δ 159.2, 143.8, 141.8, 128.9, 125.5, 119.6, 113.0, and 26.7. Anal. (C₁₈H₁₈N₆O₂) C, H, N.

20 **N, N-Diethyl-1, 4-diphenyl-1, 4-dihydro-1, 2, 4, 5-tetrazine-3, 6-dicarboxylic di-amide (5.41)**

Using ethylamine (2M solution of ethyl amine in methanol) as reactant, the title compound was prepared analogously to the synthesis of compound 5.41 in 100% yield as an orange solid, mp 239-240 °C. ¹H NMR (200MHz, CDCl₃) δ 7.40-7.13 (m, 10H), 6-53(b, 2H), 3.28 (m, 4H), 1.13 (t, J=7.4 Hz, 6H). ¹³C NMR (200MHz, CDCl₃) δ 158.1, 144.0, 141.9, 128.9, 125.4, 119.6, 115.6, 114.6, 35.1, and 14.7. Anal. (C₂₀H₂₂N₆O₂) C, H, N.

N, N-Dibutyl-1, 4-diphenyl-1, 4-dihydro-1, 2, 4, 5-tetrazine-3, 6-dicarboxylic di-amide (5.42)

30 Using butyl amine as reactant, the title compound was prepared analogously to the synthesis of compound 5.41 in 92% yield as an orange solid, mp 160-161

°C. ¹H NMR (200MHz, CDCl₃) δ 7.39-7.13 (m, 10H). 6.55(m, 2H). 3.26 (m, 4H), 2.15-1.23(m, 8H), 0.91 (t, J = 7.2 Hz, 6H). ¹³C NMR (200MHz, CDCl₃) δ 158.2, 144.0, 141.87 128.9, 125.4, 119.4, 39.8, 31.5, 20.1, and 13.8. Anal. (C₂₄H₃₀N₆O₂) C, H, N.

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N, N-Di[β-hydroxyl-α-(R)-methyl] ethyl-1, 4-diphenyl-1, 4-dihydro-1, 2, 4, 5-tetrazine-3, 6-dicarboxylic di-amide (5.43)

Using D-alaninol as reactant, the title compound 5.43 was prepared analogously to the synthesis of compound 5.41 in 94% yield as an orange solid (the product was purified by flash column chromatography with 4% ethanol-dichloromethane as eluent), mp 191-191.5 °C. ¹H NMR (200MHz, CDCl₃) δ 7.40-7.15 (m, 10H), 6.73 (d, J=7.8Hz, 2H), 4.02-3.94 (m, 2H), 3.64-3.59 (m, 2H), 3.50-3.42 (m, 2H), 2.2 (m, 2H), 1.12 (d, J=6.2Hz, 6H). ¹³C NMR (200MHz, DMSO) δ 158.2, 144.7, 141.2, 128.6, 124.3, 118.4, 63.7, 47.3, and 16.4. Anal.

15 (C₂₂H₂₆N₆O₄) C, H, N.

N, N-Diisopinocampheyl-1, 4-diphenyl-1, 4-dihydro-1, 2, 4, 5-tetrazine-3, 6-dicarboxylic di-amide (5.44) and 1, 4-Diphenyl-1, 4-dihydro-1, 2, 4, 5-tetrazine-3- carboxylic N-isopinocampheyl amide-6-carboxylic ethyl ester (5.57)

20 A solution of isopinocampheylamine (153 mg, 1 mmol) and LiHMDS (1.2 mL, 1.0 M solution in THF) in THF was stirred for 30 min at -60 °C. Compound 5.1 (76 mg, 0.2 mmol) in THF (1 mL) was added dropwise, followed by stirring for another 10 h at the same temperature. After warming up to RT. the reaction was terminated by addition of water followed by ammonium chloride aqueous solution.

25 Extracted with ethyl acetate, the organic layer was dried over sodium sulfate, filtered, and evaporated to provide the crude product. Purification on a flash column (petroleum ether:ethyl acetate 3%-10%, v/v) afforded the title compound 5.44 (60 mg, 50.4%) and 5.57 (28.5 mg, 29.3%). For; compound 5.44: mp 262-263 °C. ¹H NMR (500 MHz; DMSO:CDCl₃ = 2:1, v/v), δ (ppm): 9.03 (d, J = 8.6 Hz, 2H, NH), 7.35-7.30 (m, 8H), 7.14-7.11 (b, 2H), 4.03 (b, 2H), 2.31-2.26 (b, 4H), 1.96-1.89 (b, 4H), 1.75 (b, 2H). 1.57 (b, 2H), 1.19 (s, 6H), 1.08 (d, J = 9.4

30

Hz, 2H), 0.96 (s, 6H), 0.92 (d, J=7.2 Hz, 6H). ¹³C NMR (200 MHz, DMSO:CDCl₃=2:1, v/v). δ 158.2, 145.1, 141.3, 128.5, 124.3, 118.5, 47.4, 47.2, 43.7, 35.1, 33.7, 27.8, 23.0, and 20.5. Anal. (C₃₈H₄₆N₆O₂) C, H, N. For compound 5.57, mp 157.5-158.5 °C. ¹H NMR (500 MHz, CDCl₃). δ 7.37-7.26 (m, 8H), 7.18-7.17 (m, 2H), 6.32 (d, J=9.0 Hz, 1H, NH), 4.24 (m, 1H), 4.11 (q, J=7.2 Hz, 2H), 2.55 (b, 1H), 2.42 (b, 1H), 1.95 (b, 1H), 1.82 (b, 2H), 1.55 (b, 1H), 1.22 (s, 3H), 1.12 (d, J=7.2 Hz, 1H), 1.07 (d, J=7.1 Hz, 3H), 1.03 (t, J=7.2 Hz, 3H), 0.98 (s, 3H). ¹³C NMR (200 MHz, DMSO:CDCl₃=2:1, v/v). δ 159.7, 157.8, 141.9, 141.7, 129.3, 129.0, 125.5, 125.4, 119.4, 118.8, 111.6, 62.9, 48.7, 48.0, 46.7, 46.5, 41.7, 38.6, 35.5, 28.2, 23.5, 20.9, and 13.7. Anal. (C₂₈H₃₃N₅O₃) C, H, N.

N, N-Dipiperidinyl-1, 4-diphenyl-1, 4-dihydro-1, 2, 4, 5-tetrazine-3, 6-dicarboxylic di-amide (5.45) and 1, 4-Diphenyl-1, 4-dihydro-1, 2, 4, 5-tetrazine-3-carboxylic N-piperidinyl amide-6-carboxylic acid ethyl ester (5.58)

Using freshly distilled piperidine (from calcium hydride), the title compound was prepared analogously to 5.44 in 26% yield, mp 213-214 °C. ¹H NMR (500 MHz, CDCl₃) δ (ppm): 7.33-7.30 (m, 8H), 7.15-7.11 (m, 2H), 3.43 (m, 4H), 3.33 (m, 4H), 1.51 (m, 4H), 1.32 (m, 4H), 1.24 (m, 4H). ¹³C NMR (200 MHz, CDCl₃) δ (ppm): 159.1, 144.7, 141.3, 129.0, 125.2, 119.1, 47.5, 42.9, 26.0, 25.3, and 24.4. Anal. (C₂₆H₃₀N₆O₂) C, H, N. In the meanwhile, compound 5.58 was isolated in 12% yield. ¹H NMR (500 MHz, CDCl₃) δ (ppm): 7.36-7.33 (m, 6H), 7.25-7.21 (m, 2H), 7.18-7.14 (m, 2H), 4.13 (q, J=7.1, 2H), 3.41 (b, 2H), 3.34 (b, 2H), 1.53 (b, 2H), 1.48 (b, 4H), 1.03 (t, J=7.1 Hz, 3H). Anal. (C₂₃H₂₅N₅O₃) C, H, N.

1-(4-chlorophenyl)-4-phenyl-1, 4-dihydro-1, 2, 4, 5-tetrazine-3, 6-dicarboxylic acid diethyl ester (5.53)

Using a mixture of 5.6 and 5.12 (mole ratio = 1:1) as starting material, the title compound was prepared analogously to the synthesis of 5.28 in 20% yield. ¹H NMR (500 MHz, CDCl₃) δ (ppm): 7.37-7.18 (m, 9H), 4.19-4.09 (m, 4H),

1.12(t, J= 12 Hz, 3H), 1.00(t, J= 12 Hz, 3H). ¹³C NMR (200MHz, CDCl₃) δ (ppm): 159, 141, 140.1, 131, 129, 120, 63.2 and 13.8. Anal. (C₂₀H₁₉ClN₄O₂) C, H, N.

5 **1-(4-Chlorophenyl)-4-(4-bromophenyl)-1, 4-dihydro-1, 2, 4, 5-tetrazine-3, 6-dicarboxylic acid diethyl ester (5.54)**

Sodiohexamethyldisilazane (NaHMDS) (0.2 mL, 1 M solution in THF) was added dropwise to a solution of 5.12 (26.1 mg, 0.1 mmol) and 5.13 (30.5 mg, 0.1 mmol) in THF (0.5 mL) at -40 °C. The reaction mixture changed from light yellow
10 to reddish. After stirring for 2 h, the reaction was terminated by the addition of ammonium chloride aqueous solution, which followed by typical workup and silica gel flash column purification with petroleum ether:ethyl acetate (25: 1-10: 1, v/v) as eluent provided the title compound 5.54 as an orange solid (36 mg, 73%), mp 130-131 °C. HPLC was employed to examine the purity of 5.54: Beckman Gold
15 System; silica gel normal phase column (Phenomenex, 250 x 10 mm). two eluent systems (1) ethanol:hexane = 50:50 - 5:95 (v/v) gradient elution within 5 min, Rt = 13 min; (2) ethyl acetate:hexane = 10:90 (v/v) Rt = 19 min. Under both eluent systems, a single sharp peak was obtained. ¹H NMR (500MHz, CDCl₃) δ (ppm): 7.49 (d, J = 8.5 Hz, 2H), 7.34(d, J = 8.5Hz, 2H), 7.23(d, J= 8.5Hz, 2H), 7.17(d, J = 8.5Hz, 1H), 4.17(q, 4H), 1.10(t, J=7.0 Hz, 6H). ¹³C NMR (500MHz, CDCl₃)
20 δ (ppm): 158.9, 141.3, 141.1, 140.3, 139.8, 123.1, 129.2, 120.1, 119.8, 118.4, 63.2, and 13.6.

1-Phenyl-4-α-naphthalenyl-1, 4-dihydro-1, 2, 4, 5-tetrazine-3, 6-dicarboxylic acid diethyl ester (5.55)
25

Using 5.6 and 5.7 as starting material, the title compound was prepared analogously to the synthesis of 5.54 in 21% yield as a brown-red solid [silica gel purification with petroleum ether:ethyl acetate = 15:1 (v/v)], mp 124-125 °C. ¹H NMR (200MHz, CDCl₃) δ (ppm): 7.97-7.73 (m, 4H), 7.57-7.20 (m, 8H), 4.15 (q: J = 7.0 Hz, 2H), 3.79 (q, J = 7.4 Hz, 2H), 1.02 (t, J = 7.0 Hz, 3H), 0.63 (t, J = 7.4 Hz, 3H). ¹³C NMR (200MHz, CDCl₃) δ (ppm): 159.8, 159.2, 143.3, 141.9,
30

138.2, 134.4, 129.3, 128.5, 128.3, 126.9, 126.6, 126.4, 125.9, 125.6, 122.3, 119.6, 118.9, 63.0, 62.6, 13.8, and 13.3. Anal. ($C_{24}H_{22}N_4O_4$) C, H, N.

1, 4-Diphenyl-1, 4-dihydro-1, 2, 4, 5-tetrazine-3-aceto-6-carboxylic acid ethyl ester
5 (5.56)

Using 5.6 and 5.18 as starting material, the title compound was prepared analogously to the synthesis of 5.54 in 8.6% yield. Silica gel flash column separated the symmetric product of 5.33 (from the dimerization of 5.18) and HPLC was employed to separate the title compound from 5.1 (from the dimerization of
10 5.6). HPLC separation conditions are as follows: silica gel normal phase column (Phenomenex, 250 x 10 mm); 2.5 % ethyl acetate-hexane as eluent; R_t = 32 min for 5.54 and 35.7 min for 5.1. 1H NMR (200MHz, $CDCl_3$) δ (ppm): 7.30 (m, 1OH), 4.14 (q, J = 6.0Hz, 2H), 2.49(s, 3H), 1.04 (t, J = 7.0Hz, 3H). ^{13}C NMR (200MHz, $CDCl_3$) δ (ppm): 159.8, 41.7, 129.4, 129.2, 125.7, 125.3, 119.1, 118.9, 118.7,
15 63.1, 28.7, and 13.8.

1, 4-dihydro-1, 2, 3, 4-tetrazine-3, 6-dicarboxylic acid ester (5.62)

A three-necked flask containing 19 mL of dry ethanol was cooled to -30 °C and freshly distilled thionyl chloride (3 mL) was added dropwise. Dry dihydro-[1,
20 2, 4, 5]tetrazine-3, 6-dicarboxylic acid (2.86 g, 16.6 mmol) prepared according to the published procedure (Boger, et al, 1985) was suspended in 21 mL of dry ethanol and was added in two portions (over 15 min) to the stirred reaction mixture. The temperature was maintained at -30 °C during the additions. The reaction mixture was then allowed to warm to RT and subsequently was warmed
25 at 35-40 °C (internal temperature) for 2 h. The mixture was cooled to 0 °C internal temperature with an ice bath and the precipitate collected by filtration. The precipitate was washed with 2 mL of dry methanol and 2 mL of dry ether, and dried under vacuum. Cooling the remaining mother liquor to -30 °C afforded a second
smaller amount of product. The combined product was dissolved in anhydrous
30 methylene chloride and filtered. After removal of the solvent, the filtrate afforded the title compound as an orange solid (1.5 g, 41%), mp 110-111 °C. 1H NMR

(200MHz, CDCl_3) δ (ppm): 7.48 (s, 2H), 4.24 (q, $J = 7.2$ Hz, 4H), 1.34 (t, $J = 7.2$ Hz, 6H). Anal. ($\text{C}_8\text{H}_{12}\text{N}_4\text{O}_4$) C, H, N.

1, 4-Di-(2-iodobenzoyl)-1, 4-dihydro-1, 2, 4, 5-tetrazine-3, 6-dicarboxylic acid diethyl ester (5.64)

NaHMDS (1 mL, 1 M in THF) was added dropwise to a solution of 5.62 (114 mg, 0.5 mmol) in THF (5 mL) at -78°C , followed by the addition of 2-iodobenzoyl chloride (292.6 mg, 1.1 mmol). The reaction mixture first changed dark and then back to orange. After continuously stirring for 30 min at -78°C , the reaction was quenched by the addition of ammonium chloride aqueous solution. Standard workup and silica gel column purification (petroleum ether:ethyl acetate, 4:1. v/v) provided the title compound 5.64 (175 mg, 51%), mp $186-187^\circ\text{C}$ (recrystallization from ethyl acetate). ^1H NMR (200MHz, CDCl_3) δ 7.89 (d, $J = 7.8$ Hz, 2H), 7.45-7.32 (m, 4H), 7.18 (td, $J = 1.5$ Hz, $J = 7.6$ Hz, 2H), 4.35 (q, $J = 7.2$ Hz, 4H), 1.33 (t, $J = 7.2$ Hz, 6H). Anal. ($\text{C}_{22}\text{H}_{18}\text{I}_2\text{N}_4\text{O}_6$) C, H, N.

1, 4-Dibenzoyl-1, 4-dihydro-1, 2, 4, 5-tetrazine-3, 6-dicarboxylic acid diethyl ester (5.63) and 1-Benzoyl-1, 4-dihydro-1, 2, 4, 5-tetrazine-3, 6-dicarboxylic acid diethyl ester (5.65)

The title compound 5.63 was prepared analogously to the synthesis of 5.64 in 57% yield, and at the same time, the title compound 5.65 was isolated in 29% yield. For compound 5.63: mp $133.5-134^\circ\text{C}$. ^1H NMR (200MHz, CDCl_3) δ (ppm): 7.93-7.89 (m, 4H), 7.59-7.43 (m, 6H), 4.33 (q, $J = 7.0$ Hz, 4H), 1.29 (t, $J = 7.0$ Hz, 6H). Anal. ($\text{C}_{22}\text{H}_{20}\text{N}_4\text{O}_6$) C, H, N. For compound 5.65: mp $133-134^\circ\text{C}$. ^1H NMR (200MHz, CDCl_3) δ (ppm): 8.35 (s, 1H), 7.85 (d, $J = 6-8$ Hz, 2H), 7.54-7.41 (m, 3H), 4.36 (q, $J = 7.0$ Hz, 4H), 1.27 (t, $J = 7.0$ Hz, 6H).

Ethyl 5-oxo-2-phenyl-2, 5-dihydroisoxazole-4-carboxylate (5.68)

Hydrazine hydrate (1.7 g) was added dropwise over 30 min to a stirred suspension of 5% Rh-C (wet, 110 mg) and nitrobenzene (4.1 g) in THF (20 mL) at 15°C controlled by ice-bath. The reaction mixture was warmed to $25-30^\circ\text{C}$ for

2 h, followed by filtration. The filtrate was diluted with an equal volume of dichloromethane, dried over sodium sulfate, then condensed to a small volume. Addition of petroleum ether to this solution led to the formation of a needle-like white solid as the fairly pure product of phenyl hydroxyamine (3.2 g, 86%). The reaction mixture of phenyl hydroxyamine (2.675 g, 25 mmol), diethyl ethoxymethylene malonate (4.324 g, 20 mmol) and ethanol (20 mL) was stirred at RT for 12 h to form a large amount of solid, which after warming on a water-bath for 3 h disappeared and reappeared when the reaction mixture was cooled down to RT. Filtration and washing with ethanol provided the title compound as white crystal like solid (4.25 g, 91%). ¹H NMR (200 MHz, CDCl₃) δ (ppm): 8.40 (s, 1H), 7.46- 7.28 (m, 5H), 4.23(q, J = 7.1 Hz, 2H). 1.31 (t, J. = 7.1 Hz, 3H).

Ethyl (E)- and (Z)-2-chloro-3-phenylaminopropenoate (5.69)

The ester 5.68 (400 mg, 1.72 mmol) and triethylammonium chloride (710 mg, 5.16 mmol) were photolysed through Pyrex at 254 nm (using a 450 W Hanovia high pressure quartz mercury vapor lamp) in anhydrous acetonitrile (400 mL) under N₂ at RT. The reaction was followed by TLC (petroleum ether:ethyl acetate, 10:1, v/v) and was complete within 2h. The solvent was removed under reduced pressure, and the residue was purified by silica gel flash column chromatography (petroleum ether:ethyl acetate, 20:-10: 1, v/v) to afford the title compound (110 mg, 28.4 %). ¹H NMR (200 MHz, CDCl₃), δ (ppm): 11.02 (d, J = 13.5 Hz, 1H), 8.53 (d, J = 13.8 Hz, 1H), 7.37 (m, 2H), 7.15 (m, 3H), 4.27 (m, 2H), 1.35 (m, 3H). ¹³C NMR (200 MHz, CDCl₃), δ (ppm): 169.11, 165.76, 151-94, 139.39, 129.87, 129.33, 124.96, 117.26, 93.7, 60.37, 60.13, 14.52, and 14.41. GC-MS (EI), m/e: 225 (M⁺), 179.

1, 2, 4, 5-Tetrazine-3, 6-dicarboxylic acid diethyl ester (5.71)

An aqueous solution of sodium nitrite (10 mL, 6N) was dropped into concentrated HCl (6.26 mL). The brown gas produced was driven by nitrogen gas into a solution of 5.62 (1.07 g, 4.7 mmol) in dichloromethane (40 mL), which was cooled with an ice/water bath. The gas was bubbled directly into the stirred

reaction mixture for 15 min through a pipet. The color of the reaction mixture changed from orange to bright red during the bubbling of the NO gas. Stirring was continued for 1.5 h as the reaction mixture was warmed up to RT. The solvent and excess nitrous gases were removed under vacuum to afford the title compound
5 (991 mg, 100%) as a rose-red crystalline solid, mp 85-90 °C. ¹H NMR (200 MHz, CDCl₃) δ (ppm): 4.68 (q, J = 7.1 Hz, 4H), 1.54 (t, J = 7.1 Hz, 6H). Anal. (C₈H₁₀N₄O₄) C, H, N.

Diethyl 5H-pyridazino-[4, 5-b]-indole-1, 4-dicarboxylate (5.72)

10 To a solution of 5.71 (318 mg, 1.61 mmol) in anhydrous methylene chloride (15 mL) was added a solution of indole (100 mg, 0.85 mmol) in anhydrous methylene chloride (50 mL) dropwise over 4 h with stirring at mild reflux. Following the addition, stirring was continued for 4 h and the reaction mixture was then cooled down. After filtration, the filtrate was evaporated under (reduced
15 pressure and the residue oil was submitted to chromatography (flash column, silica gel, methylene chloride:ethyl acetate = 1:1, v/v as eluent) to afford the title compound 5.72 as an orange solid (132 mg, 52 %), mp. 151-152 °C. ¹H NMR (200 MHz, CDCl₃) δ (ppm): 11.10 (s, 1H), 8.63 (d, J = 8.2 Hz, 1H), 7.63 (d, J = 7.6 Hz, 1H), 7.50 (t, J = 7.0 Hz, 1H), 7.26 (t, J = 7.0 Hz, 1H), 4.62 (q, J = 7.0
20 Hz, 2H), 4.47 (q, J = 7.0 Hz, 2H), 1.44 (t, J = 7.0 Hz, 3H), 1.34 (t, J = 7.0 Hz, 3H). Anal. (C₁₆H₁₅N₃O₄) C, H, N.

1- Bromo-2, 5-dimethyl-4-phenylbenzene (5.74) and 1, 4-diphenyl-2, 5-dimethylbenzene (5.75)

25 Nitrogen gas was led to a solution of 2, 5-dibromo-p-xylene (845 mg, 3.2 mmol) and phenylboronic acid (800 mg, 6.8 mmol) in toluene (25 mL) for 30 min followed by the addition of Palladium tetrakis(triphenylphosphine) (360 mg, 0.31 mmol) and potassium carbonate (1.0 g). The reaction mixture was heated to 85-100 °C for 24 h, followed by the removal of the solvent under vacuum. The
30 residue was applied to silica gel flash chromatography with petroleum ether as solvent to afford the title compound 5.74 (435 mg, 52.1%) as an oil-like liquid and

5.75 (322 mg, 43.3 %) as white solid. For compound 5.74: ¹H NMR (200 MHz, CDCl₃) δ (ppm): 7.48-7.30 (m, 6H), 7.10 (s, 1H), 2.39 (s, 3H), 2.21 (s, 3H). GC-MS (EI), m/e: 262 (M⁺ + 1), 260 (M⁺ - 1), 178, 165, 152, 139, 128, 115, 102, 89, 76, 63, 51. For compound 5.75: mp 183-184 °C. ¹H NMR (200 MHz, CDCl₃) δ (ppm): 7.47-7.36 (m, 10 H), 7.16 (s, 2H), 2.28 (s; 6H). GC-MS (EI), m/e: 258 (M⁺), 241, 228, 215, 202, 189, 178, 165, 152, 115, 91, 77, 63.51.

Diethyl 2, 5-diphenyl-benzene-1, 4-dicarboxylate (5.77)

The reaction mixture of 5.75 (28 mg, 0.11 mmol), potassium permanganate (51.4 mg, 0.325 mmol), and potassium hydroxide (18.2 mg, 0.325 mmol) was heated at 100 °C for 24 h. After cooling down, the reaction mixture was filtrated and the filtrate was acidified by HCl (3N) to pH = 1, followed by the extraction with ethyl acetate. After removal of the solvent, the organic layer afforded compound 2, 5-diphenyl-1, 4-dicarboxylic acid (5.76) as a white solid (15 mg, mixed with starting material).

Dried with P₂O₅, the above crude compound 5.76 was refluxed with thionyl chloride in toluene, followed by removal of the solvent under vacuum and reaction with ethanol to provide the title compound 5.77 (3.1 mg, 7.5% overall yield from 5.75). The low yield comes from the incomplete oxidation of 5.75 to 5.76). ¹H NMR (200 MHz, CDCl₃) δ (ppm): 7.82 (s, 2H), 7.40-7.39 (m, 10H), 4.11 (q, J = 7.1 Hz, 4H), 1.00 (t, J = 7.1 Hz, 6H). GC-MS (EI), m/e: 374 (M⁺), 329, 255, 226, 215, 150, 113, 77, 51.

The prepared cannabinoid compounds were tested for CB2 receptor binding affinity and for CB1 receptor affinity (to determine selectivity for the CB2 receptor). As used herein, "binding affinity" is represented by the IC₅₀ value which is the concentration of an analog required to occupy the 50% of the total number (B_{max}) of the receptors. The lower the IC₅₀ value, the higher the binding affinity. As used herein a compound is said to have "binding selectivity" if it has higher binding affinity for one receptor compared to the other receptor; e.g. a compound that has an IC₅₀ of 0.1 nM for CB1 and 10 nM for CB2, is 100 times more selective for the CB1 receptor. The binding affinities (K_i) are expressed in nanomoles (nM).

For the CB1 receptor binding studies, membranes were prepared from rat forebrain membranes according to the procedure of P.R. Dodd et al; A Rapid Method for Preparing Synaptosomes: Comparison with Alternative Procedures, Brain Res., 107 - 118 (1981). The binding of the novel analogues to the CB1
5 cannabinoid receptor was assessed as described in W.A. Devane et al; Determination and Characterization of a Cannabinoid Receptor in a Rat Brain, Mol. Pharmacol., 34, 605 - 613 (1988) and A. Charalambous et al; "5'-azido ⁸-THC: A Novel Photoaffinity Label for the Cannabinoid Receptor", J. Med. Chem., 35, 3076 - 3079 (1992) with the following changes. The above articles are
10 incorporated by reference herein.

Membranes, previously frozen at -80 °C, were thawed on ice. To the stirred suspension was added three volumes of TME (25mM Tris-HCl buffer, 5 mM MgCl₂ and 1 mM EDTA) at a pH 7.4. The suspension was incubated at 4 °C for 30 min. At the end of the incubation, the membranes were pelleted and washed three
15 times with TME.

The treated membranes were subsequently used in the binding assay described below. Approximately 30 µg of membranes were incubated in silanized 96-well microtiter plate with TME containing 0.1% essentially fatty acid-free bovine serum albumin (BSA), 0.8 nM [³H] CP-55,940, and various concentrations of test
20 materials at 30 °C for 1 hour. The samples were immediately filtered using a Packard Filtermate 196 and Whatman GF/C filterplates and washed with wash buffer (TME) containing 0.5% BSA. Radioactivity was detected using MicroScint 20 scintillation cocktail added directly to the dried filterplates, and the filterplates were counted using a Packard Instruments Top-Count. Nonspecific binding was
25 assessed using 100 nM CP-55,940. Data collected from three independent experiments performed with duplicate determinations was normalized between 100% and 0% specific binding for [³H] CP-55,940, determined using buffer and 100 nM CP-55,940. The normalized data was analyzed using a 4-parameter nonlinear logistic equation to yield IC₅₀ values. Data from at least two independent
30 experiments performed in duplicate was used to calculate IC₅₀ values which were converted to K_i values using the assumptions of Cheng et al;

"Relationship Between the Inhibition Constant (K_i) and the concentration of Inhibitor which causes 50% Inhibition (IC_{50}) of an Enzymatic Reaction", Biochem. Pharmacol., 22, 3099-3102, (1973), which is incorporated by reference herein.

For the CB2 receptor binding studies, membranes were prepared from frozen
5 mouse spleen essentially according to the procedure of P.R. Dodd et al; "A Rapid Method for Preparing Synaptosomes: Comparison with Alternative Procedures", Brain Res., 226, 107 - 118 (1981) which is incorporated by reference herein. Silanized centrifuge tubes were used throughout to minimize receptor loss due to adsorption. The CB2 binding assay was conducted in the same manner as the CB1
10 binding assay. The binding affinities (K_i) were also expressed in nanomoles (nM). The structures, binding affinities and selectivities are summarized in Table 1.

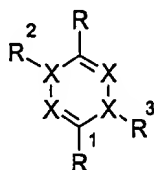
Intracellular cyclic AMP (cAMP) levels are measured with a comparative protein binding assay (materials available from Diagnostic Products, Inc. of Carlsbad, Ca.) generally according to the method described in Tao, Q. and M.E.
15 Abood; "Mutation of a highly conserved aspartate residue in the second transmembrane domain of the cannabinoid receptors, CB1 and CB2, disrupts G-protein coupling", J Pharmacol Exp Ther, 1998, 285(2): pp. 651-658, which is incorporated by reference herein. Using the above method compound 2 was found to reduce formation of cyclic AMP by inhibiting adenylate cyclase, indicating that
20 compound 2, and the inventive compounds generally, function as CB2 agonists. The IC_{50} value for Compound 2 was 8 nM.

The inventive compounds are unique in having a high affinity for the CB2 receptor and relatively little affinity for the CB1 receptor. As can be seen from Table 1, some of these compounds exhibit a high selectivity for the CB2 receptor
25 of about 2 orders of magnitude.

While preferred embodiments of the foregoing invention have been set forth for purposes of illustration, the foregoing description should not be deemed a limitation of the invention herein. Accordingly, various modifications, adaptations and alternatives may occur to one skilled in the art without departing from the spirit
30 and scope of the present invention.

What Is Claimed Is:

1. A cannabimimetic compound of Formula I below, including physiologically acceptable salts, diastereomers, enantiomers, double bond isomers or mixtures thereof:



Formula I

wherein:

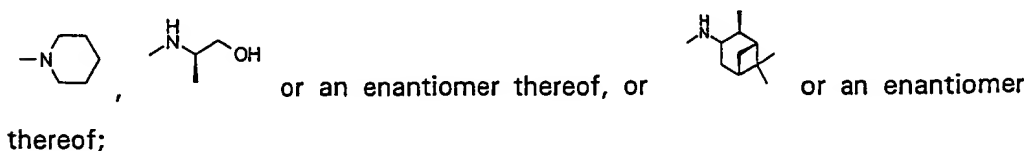
R comprises C₁₋₆alkoxy; N-alkyl; S-alkyl; C₁₋₃haloalkoxy; C₁₋₆alkylketo; C₁₋₆alkylthioketo; CO₂H; CONR⁶R⁷ where R⁶ and R⁷ each independently comprise H, lower alkyl and carbalkoxyloweralkyl; ester; thioester; reversed ester; reversed thioester; reversed amide or $\text{R}^4\text{C}(=\text{O})$ wherein R⁴ comprises

methoxy, ethoxy, propoxy, methyl, amino, methylamino, ethylamino, butylamino,



R¹ comprises C₁₋₆alkoxy; N-alkyl; S-alkyl; C₁₋₃haloalkoxy; C₁₋₆alkylketo; C₁₋₆alkylthioketo; CO₂H; CONR⁶R⁷ where R⁶ and R⁷ each independently comprise H, lower alkyl and carbalkoxyloweralkyl; ester; thioester; reversed ester; reversed thioester; reversed amide; or $\text{R}^5\text{C}(=\text{O})$ wherein R⁵ comprises

methoxy, ethoxy, propoxy, methyl, amino, methylamino, ethylamino, butylamino,

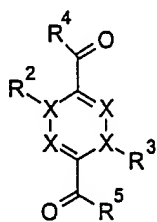


R² and R³ each independently comprise phenyl; benzyl; α-naphthyl;

methylene- α -naphthyl; β -naphthyl; methylene- β -naphthyl; 5 or 6 membered heteroaromatic rings comprising 1 to 3 heteroatoms each independently selected from N, O, and S, provided that no more than 1 heteroatom is O or S; methylene-5 or 6 membered heteroaromatic rings [correct?] comprising 1 to 3 heteroatoms each independently selected from N, O, and S, provided that no more than 1 heteroatom is O or S; any of the above comprising up to 3 substituents independently selected from halo, hydroxyl, amino, lower alkyl amino, C_{1-6} alkyl, C_{1-6} alkoxy, C_{1-6} alkylthio, CN, CF_3 , CO_2H , $CONR^6R^7$ where R^6 and R^7 each independently comprise H, lower alkyl or carbalkoxyloweralkyl, SO_3H , and $SO_2NR^6R^7$ where R^6 and R^7 each independently comprise H, lower alkyl or carbalkoxyloweralkyl; terpenes; C_{1-10} alkyl; 1,1-dimethyl alkyl or alkoxy; and

each X independently comprises CH or N.

2. The cannabimimetic compound of claim 1 having Formula II below, including physiologically acceptable salts, diastereomers, enantiomers, double bond isomers or mixtures thereof:




Formula II

wherein each X is N;

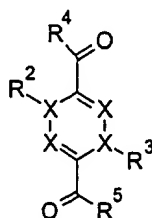
R^4 and R^5 each independently comprise methoxy, ethoxy, propoxy, methyl,

amino, methylamino, ethylamino, butylamino, , , or an enantiomer

thereof, or  or an enantiomer thereof; and

R^2 and R^3 each comprise phenyl.



3. The cannabimimetic compound of claim 1 having Formula II below, including physiologically acceptable salts, diastereomers, enantiomers, double bond isomers or mixtures thereof:



Formula II

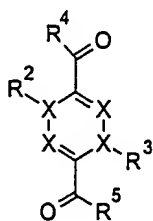
wherein each X is N;

R^4 comprises ethoxy;

R^5 comprises methyl,  or  or an enantiomer thereof; and

R^2 and R^3 each comprise phenyl.

4. The cannabimimetic compound of claim 1 having Formula II below, including physiologically acceptable salts, diastereomers, enantiomers, double bond isomers or mixtures thereof:



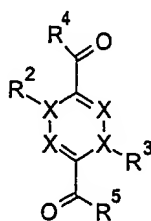
Formula II

wherein each X is N;

R^4 and R^5 each comprise ethoxy; and

R^2 and R^3 each independently comprise *p*-NO₂ substituted phenyl, *p*-Cl substituted phenyl, *p*-Br substituted phenyl, *p*-OMe substituted phenyl, *o*, *p*-dichloro substituted phenyl, 1-naphthyl or phenyl ketone.

5. The cannabimimetic compound of claim 1 having Formula II below, including physiologically acceptable salts, diastereomers, enantiomers, double bond isomers or mixtures thereof:



Formula II

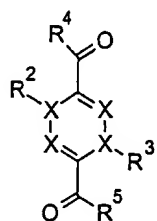
wherein each X is N;

R^4 and R^5 each comprise ethoxy;

R^2 comprises phenyl; and

R^3 comprises *p*-Cl substituted phenyl or 1-naphthyl.

6. The cannabimimetic compound of claim 1 having Formula II below, including physiologically acceptable salts, diastereomers, enantiomers, double bond isomers or mixtures thereof:



Formula II

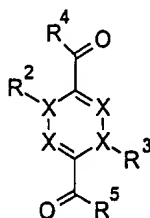
wherein each X is N;

R⁴ and R⁵ each comprise ethoxy;

R² comprises *p*-Br substituted phenyl; and

R³ comprises *p*-Cl substituted phenyl.

7. The cannabimimetic compound of claim 1 having Formula II below, including physiologically acceptable salts, diastereomers, enantiomers, double bond isomers benzene derivatives or mixtures thereof:



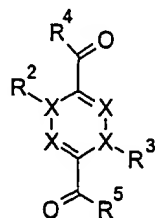
Formula II

wherein each X is CH;

R⁴ and R⁵ each comprise ethoxy; and

R² and R³ each comprise phenyl.

8. The cannabimimetic compound of claim 1 having Formula II below, including physiologically acceptable salts, diastereomers, enantiomers, double bond isomers or mixtures thereof:



Formula II

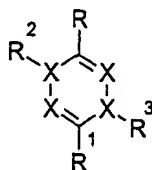
wherein each X is N;

R⁴ and R⁵ each comprise ethoxy; and

R² and R³ each comprise naphthyl.

9. The cannabimimetic compound of claim 1 wherein each X is CH and the ring structure formed thereby is a benzene derivative.


10. A pharmaceutical preparation comprising a therapeutically effective amount of a compound of Formula I below, including physiologically acceptable salts, diastereomers, enantiomers, double bond isomers or mixtures thereof:



Formula I


wherein:

R comprises C₁₋₆alkoxy; N-alkyl; S-alkyl; C₁₋₃haloalkoxy; C₁₋₆alkylketo; C₁₋₆alkylthioketo; CO₂H; CONR⁶R⁷ where R⁶ and R⁷ each independently comprise H, lower alkyl and carbalkoxyloweralkyl; ester; thioester; reversed ester; reversed

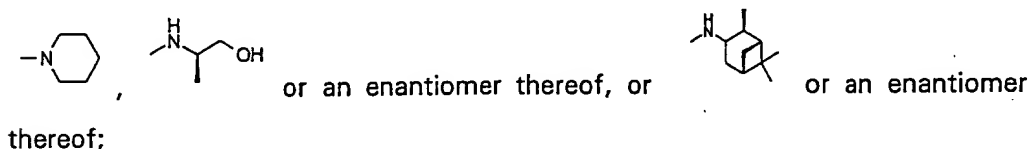
thioester; reversed amide or  wherein R⁴ comprises

methoxy, ethoxy, propoxy, methyl, amino, methylamino, ethylamino, butylamino,



R¹ comprises C₁₋₆alkoxy; N-alkyl; S-alkyl; C₁₋₃haloalkoxy; C₁₋₆alkylketo; C₁₋₆alkylthioketo; CO₂H; CONR⁶R⁷ where R⁶ and R⁷ each independently comprise H, lower alkyl and carbalkoxyloweralkyl; ester; thioester; reversed ester; reversed thioester; reversed amide; or  wherein R⁵ comprises

methoxy, ethoxy, propoxy, methyl, amino, methylamino, ethylamino, butylamino,

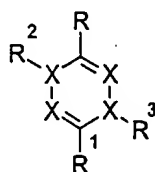


R² and R³ each independently comprise phenyl; benzyl; α-naphthyl; methylene-α-naphthyl; β-naphthyl; methylene-β-naphthyl; 5 or 6 membered heteroaromatic rings comprising 1 to 3 heteroatoms each independently selected from N, O, and S, provided that no more than 1 heteroatom is O or S; methylene-5 or 6 membered heteroaromatic rings [correct?] comprising 1 to 3 heteroatoms each independently selected from N, O, and S, provided that no more than 1 heteroatom is O or S; any of the above comprising up to 3 substituents independently selected from halo, hydroxyl, amino, lower alkyl amino, C₁₋₃alkyl, C₁₋₆alkoxy, C₁₋₆alkylthio, CN, CF₃, CO₂H, CONR⁶R⁷ where R⁶ and R⁷ each independently comprise H, lower alkyl or carbalkoxyloweralkyl, SO₃H, and SO₂NR⁶R⁷ where R⁶ and

R⁷ each independently comprise H, lower alkyl or carbalkoxyloweralkyl; terpenes; C₁₋₁₀alkyl; 1,1-dimethyl alkyl or alkoxy; and

each X independently comprises CH or N to yield either carbocyclic rings or heterocyclic rings.


11. A method of stimulating a cannabinoid receptor in an individual or animal comprising administering to the individual or animal a therapeutically effective amount of a compound of Formula I below, including physiologically acceptable salts, diastereomers, enantiomers, double bond isomers or mixtures thereof:



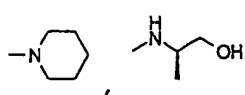
Formula I

wherein:

R comprises C₁₋₆alkoxy; N-alkyl; S-alkyl; C₁₋₃haloalkoxy; C₁₋₆alkylketo; C₁₋₆alkylthioketo; CO₂H; CONR⁶R⁷ where R⁶ and R⁷ each independently comprise H, lower alkyl and carbalkoxyloweralkyl; ester; thioester; reversed ester; reversed

thioester; reversed amide or  wherein R⁴ comprises


methoxy, ethoxy, propoxy, methyl, amino, methylamino, ethylamino, butylamino,




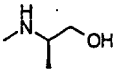

or an enantiomer thereof, or



or an enantiomer thereof;

R¹ comprises C₁₋₆alkoxy; N-alkyl; S-alkyl; C₁₋₃haloalkoxy; C₁₋₆alkylketo; C₁₋₆alkylthioketo; CO₂H; CONR⁶R⁷ where R⁶ and R⁷ each independently comprise H, lower alkyl and carbalkoxyloweralkyl; ester; thioester; reversed ester; reversed thioester; reversed amide; or  wherein R⁵ comprises

methoxy, ethoxy, propoxy, methyl, amino, methylamino, ethylamino, butylamino,

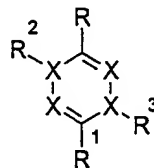
, , or an enantiomer thereof, or  or an enantiomer thereof;

R² and R³ each independently comprise phenyl; benzyl; α-naphthyl; methylene-α-naphthyl; β-naphthyl; methylene-β-naphthyl; 5 or 6 membered heteroaromatic rings comprising 1 to 3 heteroatoms each independently selected from N, O, and S, provided that no more than 1 heteroatom is O or S; methylene-5 or 6 membered heteroaromatic rings [correct?] comprising 1 to 3 heteroatoms each independently selected from N, O, and S, provided that no more than 1 heteroatom is O or S; any of the above comprising up to 3 substituents independently selected from halo, hydroxyl, amino, lower alkyl amino, C₁₋₆alkyl, C₁₋₆alkoxy, C₁₋₆alkylthio, CN, CF₃, CO₂H, CONR⁶R⁷ where R⁶ and R⁷ each independently comprise H, lower alkyl or carbalkoxyloweralkyl, SO₃H, and SO₂NR⁶R⁷ where R⁶ and R⁷ each independently comprise H, lower alkyl or carbalkoxyloweralkyl; terpenes; C₁₋₁₀alkyl; 1,1-dimethyl alkyl or alkoxy; and

each X independently comprises CH or N to yield either carbocyclic rings or heterocyclic rings.

12. A method of selectively stimulating a CB2 cannabinoid receptor in an individual or animal comprising administering to the individual or animal a therapeutically effective amount of a compound of Formula I below, including

physiologically acceptable salts, diastereomers, enantiomers, double bond isomers or mixtures thereof:

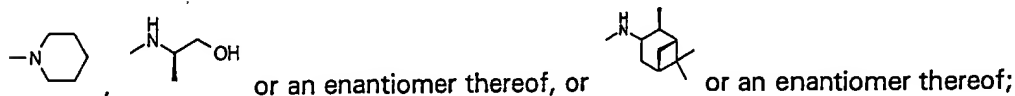


Formula I

wherein:

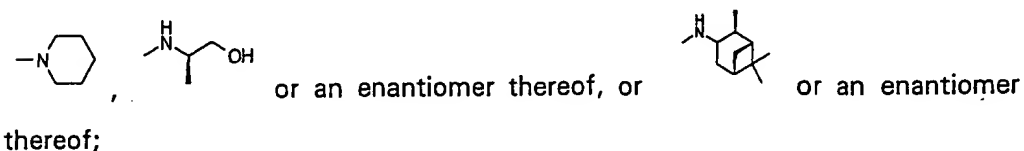
R comprises C₁₋₆alkoxy; N-alkyl; S-alkyl; C₁₋₃haloalkoxy; C₁₋₆alkylketo; C₁₋₆alkylthioketo; CO₂H; CONR⁶R⁷ where R⁶ and R⁷ each independently comprise H, lower alkyl and carbalkoxyloweralkyl; ester; thioester; reversed ester; reversed thioester; reversed amide or $\text{R}^4\text{C}(=\text{O})$ wherein R⁴ comprises

methoxy, ethoxy, propoxy, methyl, amino, methylamino, ethylamino, butylamino,



R¹ comprises C₁₋₆alkoxy; N-alkyl; S-alkyl; C₁₋₃haloalkoxy; C₁₋₆alkylketo; C₁₋₆alkylthioketo; CO₂H; CONR⁶R⁷ where R⁶ and R⁷ each independently comprise H, lower alkyl and carbalkoxyloweralkyl; ester; thioester; reversed ester; reversed thioester; reversed amide; or $\text{R}^5\text{C}(=\text{O})$ wherein R⁵ comprises

methoxy, ethoxy, propoxy, methyl, amino, methylamino, ethylamino, butylamino,

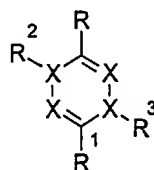


R² and R³ each independently comprise phenyl; benzyl; α-naphthyl; methylene-α-naphthyl; β-naphthyl; methylene-β-naphthyl; 5 or 6 membered heteroaromatic rings comprising 1 to 3 heteroatoms each independently selected from N, O, and S, provided that no more than 1 heteroatom is O or S; methylene-

5 or 6 membered heteroaromatic rings [correct?] comprising 1 to 3 heteroatoms each independently selected from N, O, and S, provided that no more than 1 heteroatom is O or S; any of the above comprising up to 3 substituents independently selected from halo, hydroxyl, amino, lower alkyl amino, C₁₋₆alkyl, C₁₋₆alkoxy, C₁₋₆alkylthio, CN, CF₃, CO₂H, CONR⁶R⁷ where R⁶ and R⁷ each independently comprise H, lower alkyl or carbalkoxyloweralkyl, SO₃H, and SO₂NR⁶R⁷ where R⁶ and R⁷ each independently comprise H, lower alkyl or carbalkoxyloweralkyl; terpenes; C₁₋₁₀alkyl; 1,1-dimethyl alkyl or alkoxy; and

each X independently comprises CH or N to yield either carbocyclic rings or heterocyclic rings.

13. A method of providing a physiological effect in an individual or animal comprising administering to the individual or animal a therapeutically effective amount of a compound of Formula I below, including physiologically acceptable salts, diastereomers, enantiomers, double bond isomers or mixtures thereof:




Formula I

wherein:


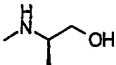

R comprises C₁₋₆alkoxy; N-alkyl; S-alkyl; C₁₋₃haloalkoxy; C₁₋₆alkylketo; C₁₋₆alkylthioketo; CO₂H; CONR⁶R⁷ where R⁶ and R⁷ each independently comprise H, lower alkyl and carbalkoxyloweralkyl; ester; thioester; reversed ester; reversed thioester; reversed amide or $R^4 = \text{C}(=\text{O})$ wherein R⁴ comprises

methoxy, ethoxy, propoxy, methyl, amino, methylamino, ethylamino, butylamino,



R¹ comprises C₁₋₆alkoxy; N-alkyl; S-alkyl; C₁₋₃haloalkoxy; C₁₋₆alkylketo; C₁₋₆alkylthioketo; CO₂H; CONR⁶R⁷ where R⁶ and R⁷ each independently comprise H, lower alkyl and carbalkoxyloweralkyl; ester; thioester; reversed ester; reversed thioester; reversed amide; or  wherein R⁵ comprises

methoxy, ethoxy, propoxy, methyl, amino, methylamino, ethylamino, butylamino,

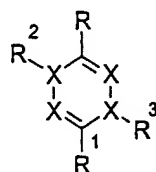
,  or an enantiomer thereof, or  or an enantiomer thereof;

R² and R³ each independently comprise phenyl; benzyl; α-naphthyl; methylene-α-naphthyl; β-naphthyl; methylene-β-naphthyl; 5 or 6 membered heteroaromatic rings comprising 1 to 3 heteroatoms each independently selected from N, O, and S, provided that no more than 1 heteroatom is O or S; methylene-5 or 6 membered heteroaromatic rings [correct?] comprising 1 to 3 heteroatoms each independently selected from N, O, and S, provided that no more than 1 heteroatom is O or S; any of the above comprising up to 3 substituents independently selected from halo, hydroxyl, amino, lower alkyl amino, C₁₋₆alkyl, C₁₋₆alkoxy, C₁₋₆alkylthio, CN, CF₃, CO₂H, CONR⁶R⁷ where R⁶ and R⁷ each independently comprise H, lower alkyl or carbalkoxyloweralkyl, SO₃H, and SO₂NR⁶R⁷ where R⁶ and R⁷ each independently comprise H, lower alkyl or carbalkoxyloweralkyl; terpenes; C₁₋₁₀alkyl; 1,1-dimethyl alkyl or alkoxy; and

each X independently comprises CH or N to yield either carbocyclic rings or heterocyclic rings.

14. A method of treating a condition in an animal or individual selected from pain, peripheral pain, glaucoma, epilepsy and nausea such as associated with cancer chemotherapy; cancer; a neurodegenerative disease, or of reducing fertility; or of

reducing a disease associated with motor function; or of reducing inflammation; or of providing neuroprotection; or of modulating the immune system; or any combination thereof, comprising administering to the individual or animal in need of such treatment an amount of a compound of Formula I below, including physiologically acceptable salts, diastereomers, enantiomers, double bond isomers or mixtures thereof:



Formula I

wherein:

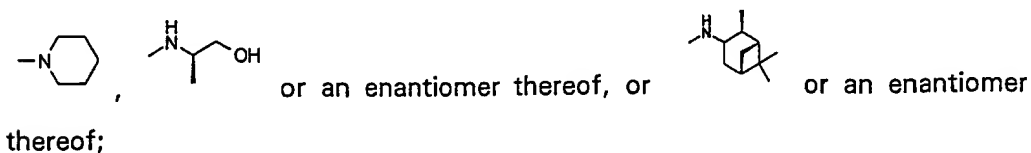
R comprises C₁₋₆alkoxy; N-alkyl; S-alkyl; C₁₋₃haloalkoxy; C₁₋₆alkylketo; C₁₋₆alkylthioketo; CO₂H; CONR⁶R⁷ where R⁶ and R⁷ each independently comprise H, lower alkyl and carbalkoxyloweralkyl; ester; thioester; reversed ester; reversed thioester; reversed amide or $R^4 = \text{C}(=\text{O})\text{R}^4$ wherein R⁴ comprises

methoxy, ethoxy, propoxy, methyl, amino, methylamino, ethylamino, butylamino,



R¹ comprises C₁₋₆alkoxy; N-alkyl; S-alkyl; C₁₋₃haloalkoxy; C₁₋₆alkylketo; C₁₋₆alkylthioketo; CO₂H; CONR⁶R⁷ where R⁶ and R⁷ each independently comprise H, lower alkyl and carbalkoxyloweralkyl; ester; thioester; reversed ester; reversed thioester; reversed amide; or $R^5 = \text{C}(=\text{O})\text{R}^5$ wherein R⁵ comprises

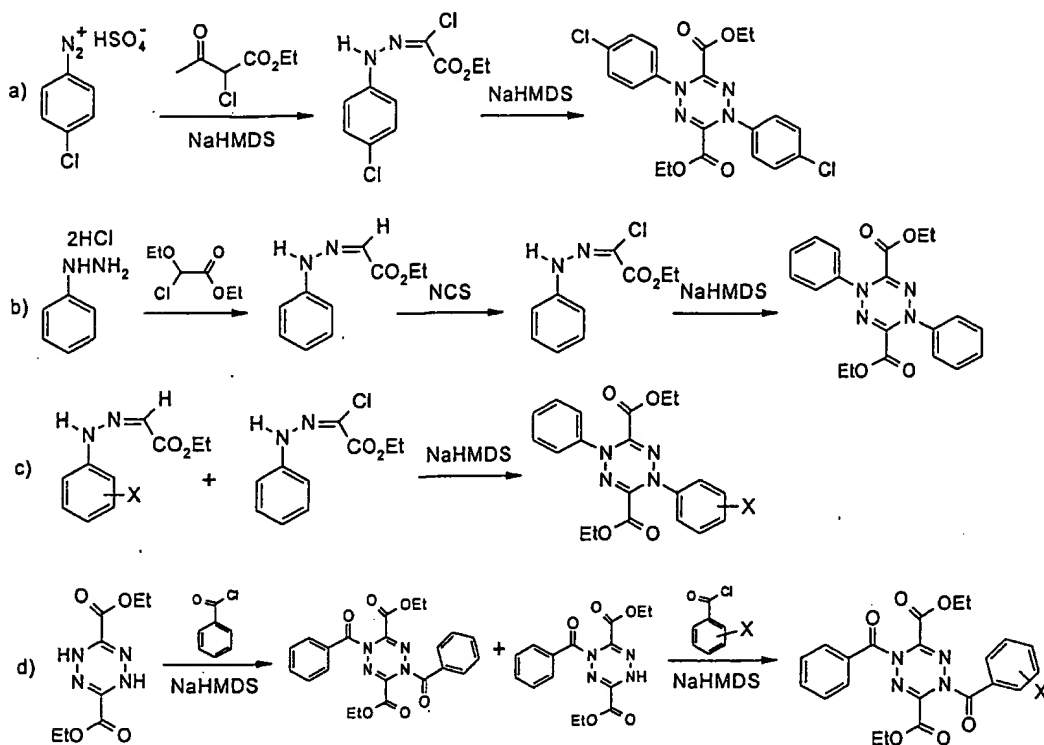
methoxy, ethoxy, propoxy, methyl, amino, methylamino, ethylamino, butylamino,



R^2 and R^3 each independently comprise phenyl; benzyl; α -naphthyl; methylene- α -naphthyl; β -naphthyl; methylene- β -naphthyl; 5 or 6 membered heteroaromatic rings comprising 1 to 3 heteroatoms each independently selected from N, O, and S, provided that no more than 1 heteroatom is O or S; methylene-5 or 6 membered heteroaromatic rings [correct?] comprising 1 to 3 heteroatoms each independently selected from N, O, and S, provided that no more than 1 heteroatom is O or S; any of the above comprising up to 3 substituents independently selected from halo, hydroxyl, amino, lower alkyl amino, C_{1-6} alkyl, C_{1-6} alkoxy, C_{1-6} alkylthio, CN, CF_3 , CO_2H , $CONR^6R^7$ where R^6 and R^7 each independently comprise H, lower alkyl or carbalkoxyloweralkyl, SO_3H , and $SO_2NR^6R^7$ where R^6 and R^7 each independently comprise H, lower alkyl or carbalkoxyloweralkyl; terpenes; C_{1-10} alkyl; 1,1-dimethyl alkyl or alkoxy; and

each X independently comprises CH or N to yield either carbocyclic rings or heterocyclic rings.

15. A method of preparing a tetrazine analog, comprising any of routes a - d:



16. The method of claim 15 wherein the prepared tetrazine analog comprises a cannabinoid compound.

17. The method of claim 15 wherein the prepared tetrazine analog comprises a CB2 selective cannabinoid compound.